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MAJOR ARTICLE

HIV RNA Screening Reduces Integrase Strand Transfer Inhibitor Resistance Risk in Persons Receiving Long-Acting Cabotegravir for HIV Prevention

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Background. The HPTN 083 trial demonstrated that long-acting cabotegravir (CAB-LA) was superior to tenofovir-disoproxil fumarate/emtricitabine for human immunodeficiency virus (HIV) preexposure prophylaxis (PrEP). Integrase strand transfer inhibitor (INSTI) resistance-associated mutations (RAMs) were detected in some participants with HIV infection. We used a low viral load INSTI genotyping assay to evaluate the timing of emergence of INSTI RAMs and assessed whether HIV screening with a sensitive RNA assay would have detected HIV infection before INSTI resistance emerged.

Methods. Single-genome sequencing to detect INSTI RAMs was performed for samples with viral loads <500 copies/mL from 5 participants with previously identified INSTI RAMs and 2 with no prior genotyping results.

Results. Major INSTI RAMs were detected in all 7 cases. HIV RNA testing identified infection before major INSTI RAMs emerged in 4 cases and before additional major INSTI RAMs accumulated in 1 case. Most INSTI RAMs were detected early when the viral load was low and CAB concentration was high.

Conclusions. When using CAB-LA PrEP, earlier detection of HIV infection with a sensitive RNA assay may allow for earlier treatment initiation with the potential to reduce INSTI resistance risk. Further studies are needed to evaluate the value and feasibility of HIV RNA testing with CAB-LA PrEP.

Keywords. HIV; HPTN; INSTI; cabotegravir; injectable; integrase; PrEP; prevention; resistance.

The HIV Prevention Trials Network (HPTN) 083 trial compared the efficacy of long-acting injectable cabotegravir (CAB-LA) to daily oral tenofovir disoproxil fumarate/emtricitabine (TDF/FTC) for human immunodeficiency virus (HIV) preexposure prophylaxis (PrEP) [1]. The trial enrolled cisgender men and transgender women who have sex with men in the United States, South America, Africa, and Asia. The trial demonstrated that CAB-LA was superior to TDF/FTC for HIV prevention [1]. In December 2021, CAB-LA was approved by the US Food and Drug Administration (FDA) to reduce the risk of sexually acquired HIV-1 infection [2].

HIV infections in the CAB-LA arm were rare in HPTN 083. In the blinded phase of the trial, only 16 infections were detected among 2282 participants with 3205 person-years of follow-up (4 baseline, 12 incident; incidence, 0.41 per 100 person-years) [1]. In many of these cases, retrospective testing showed that viral replication was suppressed for long periods and anti-HIV antibody production was significantly delayed and diminished [3]. Consequently, detection of these infections was often delayed at study sites where HIV rapid tests and a laboratory-based antigen/antibody (Ag/Ab) assay were used to screen for HIV infection [3]. In some cases, delayed HIV diagnosis led to provision of CAB in persons with undetected HIV infection, delayed antiretroviral treatment (ART) initiation, and emergence of resistance to integrase strand transfer inhibitors (INSTIs). Delayed HIV diagnosis also has potential to impact personal health and on-going HIV transmission. Our prior studies demonstrated that infections in persons with recent CAB exposure were detected earlier using sensitive HIV RNA testing [3, 4].

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Use of any antiretroviral drug for HIV prevention has the potential to lead to resistance if baseline infections are not detected before starting PrEP or if infection occurs after PrEP initiation. This could potentially compromise use of the specific PrEP agent or other drugs in the same drug class when used as a component of a subsequent HIV treatment regimen [5, 6]. We previously evaluated INSTI resistance in the 16 cases of HIV infection that were identified in the CAB arm of the trial before study unblinding [3]. In that study, HIV genotyping was performed for samples with viral loads >500 copies/mL. INSTI resistance associated mutations (RAMs) were detected in 5 cases. Testing was not performed in 2 cases because the viral load was <500 copies/mL at all study visits [3].

In this report, we used a low viral load INSTI genotyping assay to analyze samples from the 5 cases noted above with INSTI resistance and the 2 cases with no prior resistance results. This provided additional information on the timing of emergence of INSTI RAMs. We used these data to evaluate whether use of a sensitive RNA assay for HIV screening in persons receiving CAB-LA PrEP would allow for ART initiation before INSTI RAMs emerged, potentially reducing INSTI resistance risk. This is of particular relevance to use of CAB-LA in the United States because the US FDA prescribing information specifies use of an RNA assay for HIV screening with CAB-LA PrEP [2].

METHODS

HPTN 083 Study Design

Information on the design and outcomes of HPTN 083 (NCT02720094) is presented in the primary study report [1]. Participants randomized to the CAB arm received 5 weeks of 30 mg of oral CAB daily, followed by CAB-LA injections of 600 mg of CAB as a single intramuscular injection in the gluteal region for approximately 3 years (2 injections 1 month apart followed by injections every 2 months). Participants who discontinued CAB injections early or completed all scheduled CAB injections were offered daily oral TDF/FTC PrEP for 48 weeks. The blinded phase of the trial was stopped early by a Data Safety Monitoring Board when trial data demonstrated that CAB-LA PrEP was highly effective. This report includes data for visits that occurred in the blinded phase of the trial (prior to 15 May 2020).

Testing at Study Sites

HIV testing was performed at study sites as previously described [3]. Participants were required to have a negative HIV RNA test within 14 days before study enrollment. At enrollment and follow-up visits, participants were screened for HIV infection using 1 or 2 HIV rapid tests and an FDA-cleared laboratory-based Ag/Ab test. Locally available tests were used to confirm infection; an ultrasensitive HIV DNA test was performed at Johns Hopkins University (Baltimore, MD) in real time in selected cases.

Retrospective Testing

Retrospective testing was performed at the HPTN Laboratory Center as described [3]. This testing included confirmation of HIV infection, determination of the timing of HIV infection, viral load testing, HIV resistance testing, HIV subtyping, and quantification of CAB concentrations. Viral load testing was performed using the RealTime HIV-1 Viral Load Assay (Abbott Molecular; lower limit of quantification, 40 copies/mL); selected samples were also analyzed using a single-copy RNA assay [7]. HIV genotyping was performed previously using the Genosure PRIme assay (Monogram Biosciences) for samples with viral loads >500 copies/mL. CAB concentrations were determined by liquid chromatography-tandem mass spectrometry. In this report, INSTI genotyping was performed for samples that had a positive result with the APTIMA HIV-1 RNA Qualitative assay (Hologic; lower limit of detection, 30 copies/mL) and had <500 HIV RNA copies/mL (see below). The Stanford HIV Drug Resistance Database [8] was used to classify INSTI RAMs detected by the GenoSure PRIme and low viral load single-genome integrase sequencing (low VL SGS-IN) assays as accessory mutations or major INSTI RAMs. HIV subtyping was performed at Monogram Biosciences for samples analyzed with the GenoSure PRIme assay [3].

Classification of HIV Infections

Results from HIV testing performed at study sites and the HPTN Laboratory Center were reviewed by an independent committee, which determined HIV infection status and the timing of HIV infection [3]. Two key visits were identified for each case. The first HIV-positive visit was identified based on results of retrospective HIV RNA testing performed at the HPTN Laboratory Center [3]. The first site positive visit is the first visit where the participant had a reactive rapid test or a reactive Ag/Ab test at the study site [3]. Infections were classified based on the timing of HIV infection: (1) baseline infections, (2) infections with no recent CAB exposure, (3) oral-phase infections, and (4) infections that occurred despite on-time CAB-LA injections [3].

Low Viral Load INSTI genotyping

Single-genome sequencing of a portion of HIV *pol* encoding the entire integrase protein was performed at the University of Pittsburgh using the low VL SGS-IN assay as described previously [9, 10]; 1–1.5 mL of plasma was used for testing. Testing involved extraction of HIV RNA; reverse transcription to generate cDNA from the HIV integrase coding region; end point dilution of cDNA to a single HIV template per polymerase chain reaction (PCR) reaction as determined by Poisson

distribution statistics; amplification of cDNA with the generation of a 0.95-kb reverse transcription-polymerase chain reaction (RT-PCR) amplicon; and sequencing of amplicons by SGS. Primers used for amplification were selected based on known HIV subtype or likely HIV subtype based on the geographical location of the study site. Gel Red readouts were followed by 1% agarose/1 × SB gel electrophoresis for size confirmation of PCR products. Bidirectional sequencing of amplicons by the Sanger method was performed for 8–119 independent PCR reactions per sample. Primers used in the low VL SGS-IN assay are described in Supplementary Material File 1. The following modifications were used for samples with <300 HIV RNA copies/mL: all of the extracted RNA was used for cDNA synthesis; cDNA was diluted 1:2 in 5 mM Tris-HCl; all of the diluted cDNA was used to seed multiple PCR reactions.

Sequence alignments and phylogenetic analyses of integrase sequences were performed as reported previously [10]. Briefly, sequences were aligned to the LAI reference strain; quality control was performed on sequences; sequences were trimmed to an appropriate length; references were removed; and Contigs were generated using Sequencher version 5.0 (Gene Codes). Phylogenetic analysis showed clustering of sequences from each participant with no evidence of cross-contamination (Supplementary Material File 1). GenBank accession numbers of the sequences generated by the low VL SGS-IN assay are OP539111–OP539272.

Interpretation of CAB Concentrations

The CAB-LA regimen used in HPTN 083 was meant to achieve CAB concentrations above the in vitro protein-adjusted concentration required for 90% viral inhibition (≥1 × PA-IC_{90}; 0.166 μg/mL) in 95% of individuals, ≥4 × PA-IC_{90} (0.664 μg/mL) in 80% of individuals, and ≥8 × PA-IC_{90} (1.33 μg/mL) in 50% of individuals. Median CAB concentrations ≥8 × PA-IC_{90} have been associated with viral suppression in treatment studies [11]. However, CAB pharmacologic correlates of protection against HIV acquisition have not been characterized in humans; efforts are ongoing to determine target protective concentrations for PrEP.

Ethical Considerations

The HPTN 083 protocol was approved by the institutional review boards and/or ethics committees and ministries of health for all participating sites [1]. All participants provided written informed consent.

RESULTS

Study Cohort

This report describes further characterization of 7 cases in HPTN 083 using the low VL SGS-IN genotyping assay (see "Methods"). INSTI RAMs were previously detected using the GenoSure PRIme assay at 1 or more study visits in 5 of the 7 cases; genotyping was not performed using this assay in 2 cases because the viral load was <500 copies/mL at all study visits [3] (Figure 1). In 1 case (A2), the participant had HIV infection at study enrollment that was not detected by HIV RNA testing performed at the study site at the screening visit. In 2 cases (C1, C3), HIV infection occurred during the oral lead-in phase. In the remaining 4 cases (D1–D4), HIV infection occurred in the setting of on-time CAB injections. Detection of HIV infection at the study sites using HIV rapid tests and an instrumented Ag/Ab assay was delayed in all 7 of these cases (median time after the first HIV-positive visit, 60 days; range, 35–117 days). A summary of clinical and laboratory data for each case is provided in Supplementary Material File 2.

Analysis of INSTI resistance Mutations

Twenty-one samples from the 7 cases were analyzed using the low VL SGS-IN assay. Samples selected for testing had a positive qualitative RNA assay (APTIMA HIV-1 RNA Qualitative Test, limit of detection, 30 copies/mL) and a viral load <500 copies/mL. Results were obtained for 18 of the 21 samples tested; those data were combined with data obtained previously from the GenoSure PRIme assay (Figure 2, Figure 3, Figure 4, and Table 1). One or more major INSTI RAM was detected in all 7 cases. Genotyping results were obtained at the first HIV-positive visit in 5 of the 7 cases (testing failed in 2 cases); a major INSTI RAM was detected in 1 of the 5 samples with genotyping results. Genotyping results were obtained at the first site positive visit in 6 of the 7 cases (testing was not performed for case D2 because the qualitative RNA assay was negative at that visit); 1 or more major INSTI RAMs were detected in all 6 of those samples. Additional data from the low VL SGS-IN assay is provided in Supplementary Material File 1.

In addition to testing the 7 cases described above, low VL SGS-IN testing was also performed for selected samples from HIV infections identified in the blinded phase of HPTN 083 that had no major INSTI RAMs detected with the GenoSure PRIme assay. This included 3 additional baseline cases (A1, A3, and A4) and 1 oral-phase case (C2); samples were not tested for the 5 cases with no recent CAB-LA exposure because all of the relevant samples had viral loads >500 copies/mL. No major INSTI RAMs were detected with the low VL SGS-IN assay in these cases.

Characteristics of Infections at the First Visit With a Major INSTI RAM Detected

Table 2 shows the characteristics for each case at the first visit where a major INSTI RAM was detected. In 6 cases, INSTI RAMs were first detected close to the time of the first HIV-positive visit (median 38 days, range 0–62 days); in the seventh case (D2), genotyping results were not obtained near

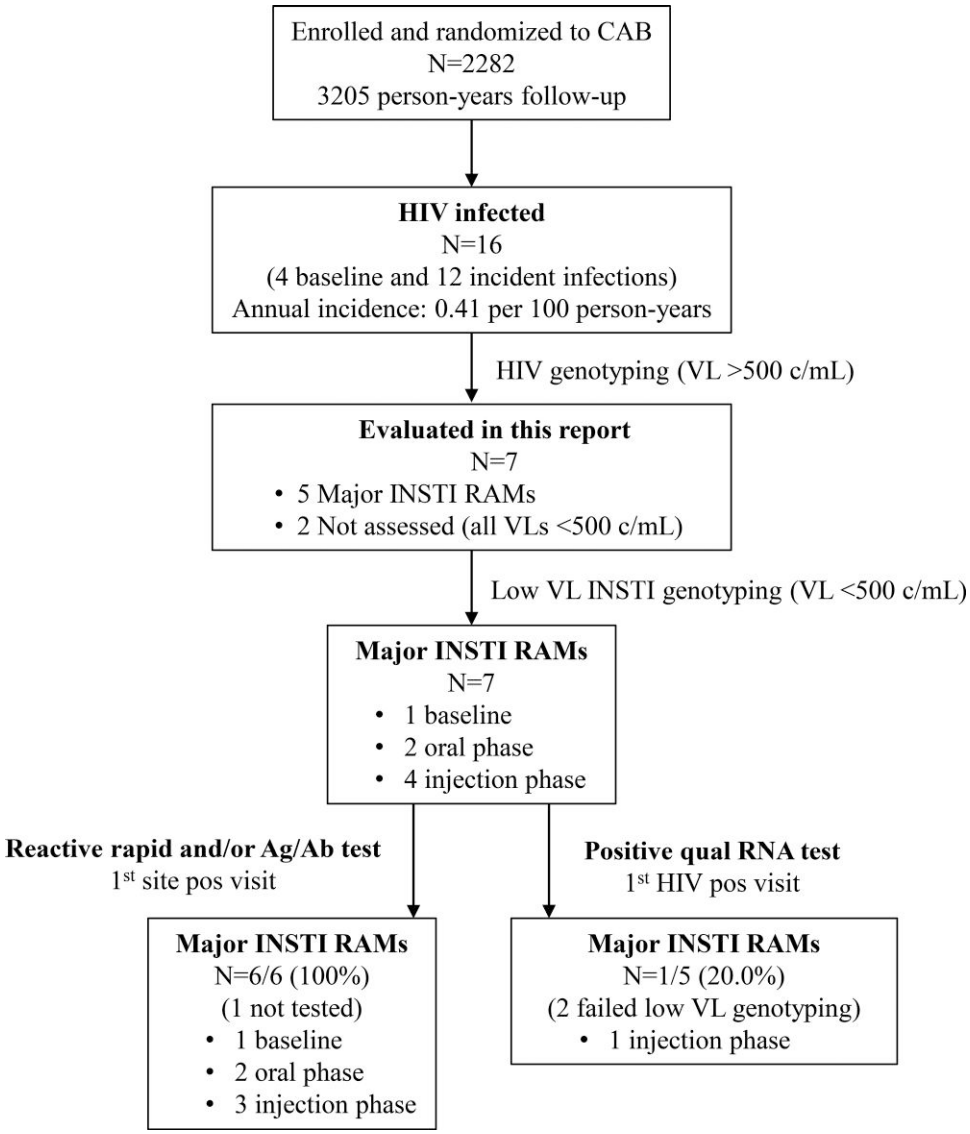

Figure 1. Study cohort and summary of findings. The top box shows the number of persons enrolled and the person-years of follow-up for the CAB arm of HPTN 083. The second box shows the number of HIV infections identified in the blinded phase of the study and the annual incidence of HIV infection in the CAB arm [1]. The third box shows the number of participants evaluated in this report. The fourth box shows how many participants had major INSTI RAMs detected at any study visit, among the 16 CAB cases analyzed to date; this box also shows the breakdown of cases by case type (baseline, oral phase, injection phase). The lower row of boxes shows the number of cases and break down of cases by case type with major INSTI RAMs at the first visit where the site detected infection based on results from HIV rapid tests and an instrumented HIV antigen/antibody test (first site positive visit) and at the first HIV positive visit. Abbreviations: Ab, antibody; Ag, antigen; c/mL, copies per mL; CAB, cabotegravir; HIV, human immunodeficiency virus; INSTI, integrase strand transfer inhibitor; RAM, resistance-associated mutation; VL, viral load.

the time of this visit because the RNA level was too low. In 6 cases, the viral load was <250 copies/mL when major INSTI RAMs were first detected (<40 copies/mL in 2 cases). In 6 cases, CAB concentrations at this visit were near or above the $8 \times$ PA-IC_{90} (observed CAB concentrations, 1.108 to 3.318 μg/mL). In the remaining case (D2), the CAB concentration was lower (0.703 μg/mL), but was $\geq 3 \times$ PA-IC_{90}, which was the plasma threshold associated with rectal protection in nonhuman primate intrarectal challenge studies [12]. In this case, it was not possible to determine if INSTI resistance was present at earlier visits with higher CAB concentrations because RNA levels were too low for HIV genotyping at most visits and the Low VL SGS-IN assay failed at the 2 visits.

Impact of Early Detection of HIV Infection on INSTI Resistance Risk

Major INSTI RAMs were already present in all 6 evaluable cases at the first site positive visit where HIV screening was performed using rapid tests and Ag/Ab tests. In contrast, use of a qualitative HIV RNA test detected all but 1 of these infections before major INSTI RAMs emerged (Figure 1). In case A2, HIV RNA was

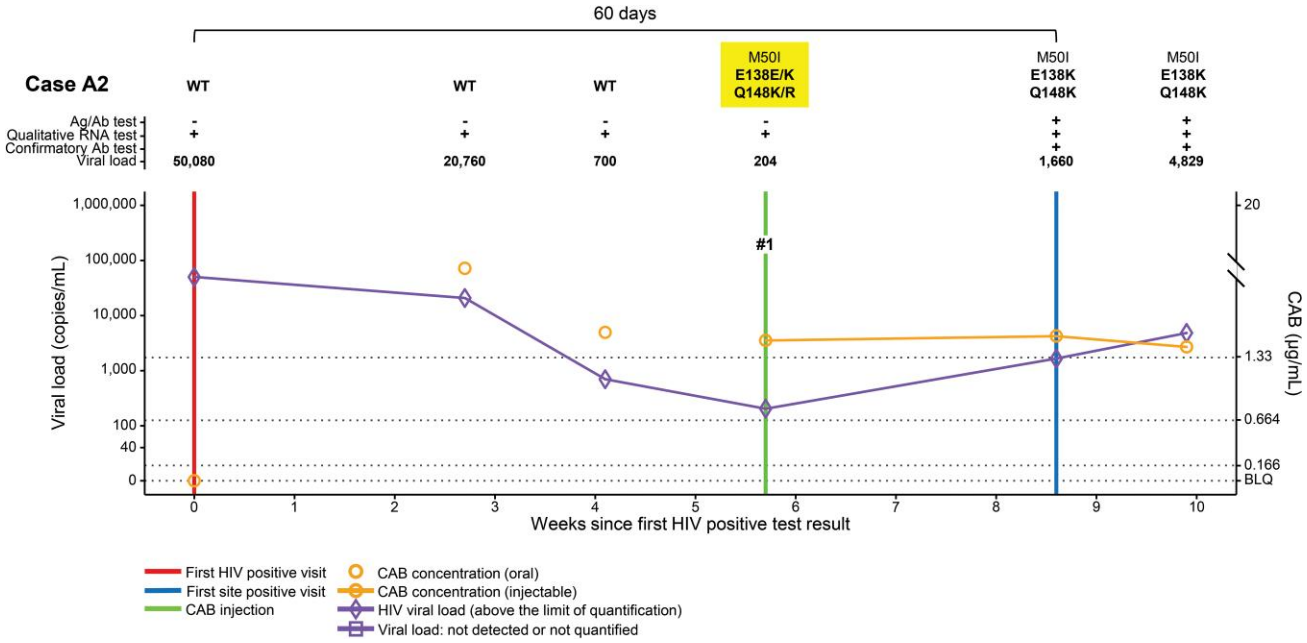

Figure 2. Case summary: baseline infection (case A2). Key events and laboratory results for participant A2. A case description is provided in Supplementary File 2. Red line indicates the first HIV positive visit; blue line indicates the first site positive visit; bracket shows the number of days between these 2 visits. Green lines indicate CAB injections. The X-axis indicates the number of weeks since the first HIV-positive test result, the left Y-axis indicates viral load, and the right Y axis indicates CAB concentration. Horizontal lines indicate CAB cutoffs (1.33 µg/mL = 8 × PA-IC_{90}; 0.664 µg/mL = 4 × PA-IC_{90}; 0.166 µg/mL = 1 × A-IC_{90}; BLQ <0.025 µg/mL). A target concentration >8× PA-IC_{90} was used to interpret drug concentrations. Results from retrospective testing performed at the HIV Prevention Trials Network Laboratory Center are shown above the graph: + reactive or positive test result, − nonreactive or negative test result. Viral load values are shown (HIV RNA copies/mL). HIV genotyping results are shown at the top; results from the low viral load single-genome integrase sequencing (low VL SGS-IN) assay are highlighted. All INSTI RAMs are shown; major INSTI RAMs are bolded. Abbreviations: Ag/Ab, antigen/antibody test; BLQ, below limit of quantification; CAB, cabotegravir; HIV, human immunodeficiency virus; INSTI, integrase strand transfer inhibitor; PA-IC_{90}, in vitro protein-adjusted 90% CAB inhibitory concentration; WT, wild type.

detected at study enrollment, before major INSTI RAMs were detected. In 3 cases (C1, C3, D3), RNA was detected at a study visit before major INSTI RAMs were detected. In 1 case (D1), RNA major INSTI RAMs were already present at the first HIV positive visit; in this case, RNA testing detected infection before additional major INSTI RAMs accumulated. In the remaining 2 cases (D2, D4), the impact of RNA screening on INSTI resistance could not be assessed because HIV genotyping results were not obtained near the time of infection.

DISCUSSION

We previously demonstrated that sensitive HIV RNA testing is more likely to detect HIV infection in the setting of CAB-LA PrEP compared to HIV screening with HIV rapid tests and Ag/Ab tests alone [3]. Here, we demonstrate that HIV screening with a sensitive RNA assay would have identified infections in most cases before major INSTI RAMs were detected or before additional major INSTI RAMs accumulated. Earlier detection of infection in this setting would provide an opportunity for earlier ART initiation. In some cases, this might allow for ART initiation prior to emergence of INSTI RAMs. This could also potentially reduce the risk for on-going transmission of INSTI-resistant HIV. Reducing INSTI resistance risk is critical because the US Department of Health and Human Services, World Health Organization, and other guidelines currently recommend INSTI-based regimens for first-line ART [5, 13, 14].

These findings support US FDA prescribing information and recent guidance from the US Centers for Disease Control and Prevention (CDC), which recommend use of RNA assay for HIV screening with CAB-LA PrEP. The US FDA prescribing information indicates that HIV RNA testing should be performed prior to initiation of CAB-LA and at every injection visit using an assay that is FDA-approved or cleared for diagnosis of acute or primary HIV infection [2]. Recent guidance from the US CDC recommends HIV RNA testing using the most sensitive assay available within 1 week before starting CAB-LA PrEP, at every injection visit, and quarterly for 12 months after stopping injections [13]. In HPTN 083, HIV RNA testing was performed within 14 days before study enrollment. Despite this testing, 7 participants were enrolled and began study products who were later determined to have acute HIV infection at study enrollment (4 in the CAB arm; 3 in the TDF/FTC arm) [3]. HIV RNA testing closer to the time of PrEP initiation may help identify persons with acute infection before PrEP is started. HIV loads are often very low in

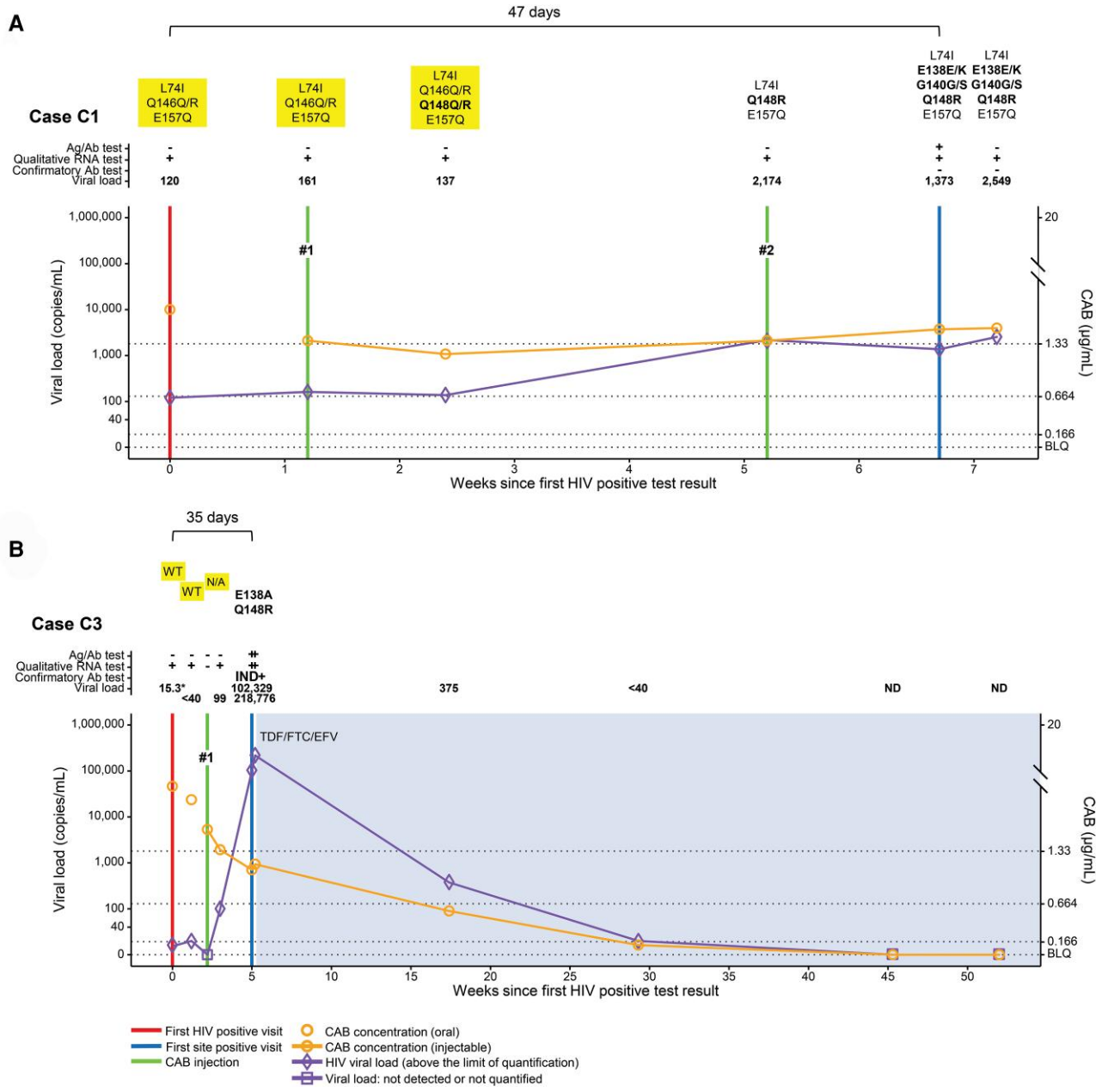

Figure 3. Case summaries: oral-phase infections (cases C1 and C3). Results for participants who acquired HIV infection while receiving oral cabotegravir: (*A*) case C1, and (*B*) case C3. Key features of the figure are as in Figure 2. Viral load values <40 indicate that HIV RNA was detected below the lower limit of quantification. One viral load result was obtained with a single-copy RNA assay (asterisk, case C3). Abbreviations: Ag/Ab, antigen/antibody test; BLQ, below limit of quantification; CAB, cabotegravir; EFV, efavirenz; FTC,emtricitabine; HIV, human immunodeficiency virus; IND, indeterminate; ND, not detected; TDF, tenofovir disoproxil fumarate; WT, wild type.

persons who acquire HIV infection once CAB-LA PrEP is initiated [3]. To be effective, HIV RNA screening during CAB-LA PrEP should be performed using a highly sensitive assay. The ongoing open-label extension for HPTN 083 is evaluating the performance of an HIV RNA test with a lower limit of quantification of 50 copies/mL or lower.

In 4 of the cases described in this report, major INSTI RAMs were clearly acquired due to CAB exposure. In 1 case (D1), a major INSTI RAM was detected at the first HIV-positive visit; all HIV tests were negative 42 days earlier. In this case, it is most likely that the INSTI RAM detected at the first HIV-positive visit was selected by CAB exposure shortly after infection. Transmitted INSTI resistance is uncommon [15, 16]. However, it is also possible that this participant had HIV infection at an earlier visit that was not detected with a qualitative RNA assay. In 2 other cases (D2, D4), it was not possible to

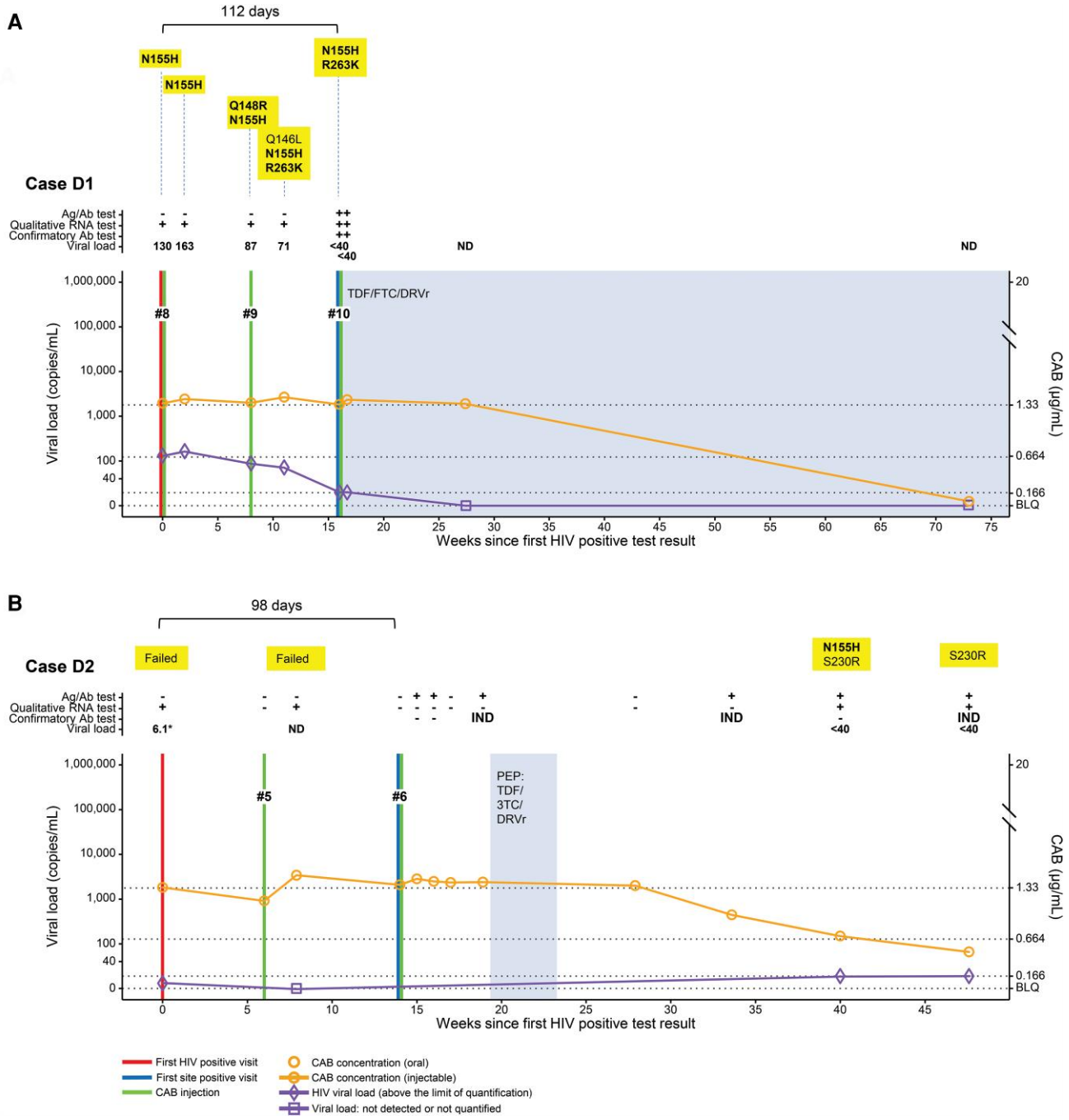

Figure 4. Case summaries: injection-phase infections (cases D1–D4). Key events and laboratory results for participants who acquired HIV infection while receiving CAB injections: (*A*) case D1, (*B*) case D2, (*C*) case D3, and (*D*) case D4. Key features of the figure are as in Figure 2. Viral load values <40 indicate that HIV RNA was detected below the lower limit of quantification. One viral load result was obtained with a single-copy RNA assay (asterisk, case D2). The nonnucleoside reverse transcriptase inhibitor resistance mutation, K103N, is shown in blue text (case D3). Abbreviations: Ag/Ab, antigen/antibody test; BLQ, below limit of quantification; CAB, cabotegravir; DRVc, darunavir/cobicistat; DRVr, darunavir/ritonavir; EFV, efavirenz; FTC, emtricitabine; HIV, human immunodeficiency virus; IND, indeterminate; ND, not detected; PEP, postexposure prophylaxis; TAF, tenofovir alafenamide; TDF, tenofovir disoproxil fumarate.

determine whether the participant had INSTI resistance at the first HIV-positive visit because genotyping results were not obtained. In these 2 cases, the qualitative RNA was positive at the first HIV-positive visit even though the viral loads were below the lower limit of detection of this assay (6.1 and 15.3 copies/mL, results obtained using a single-copy RNA assay); in these cases, the qualitative assay was negative at some later visits. This highlights the challenges of detecting HIV infection, determining the timing of infection, and evaluating INSTI resistance in the setting of CAB-LA PrEP. Use of total nucleic acid tests that

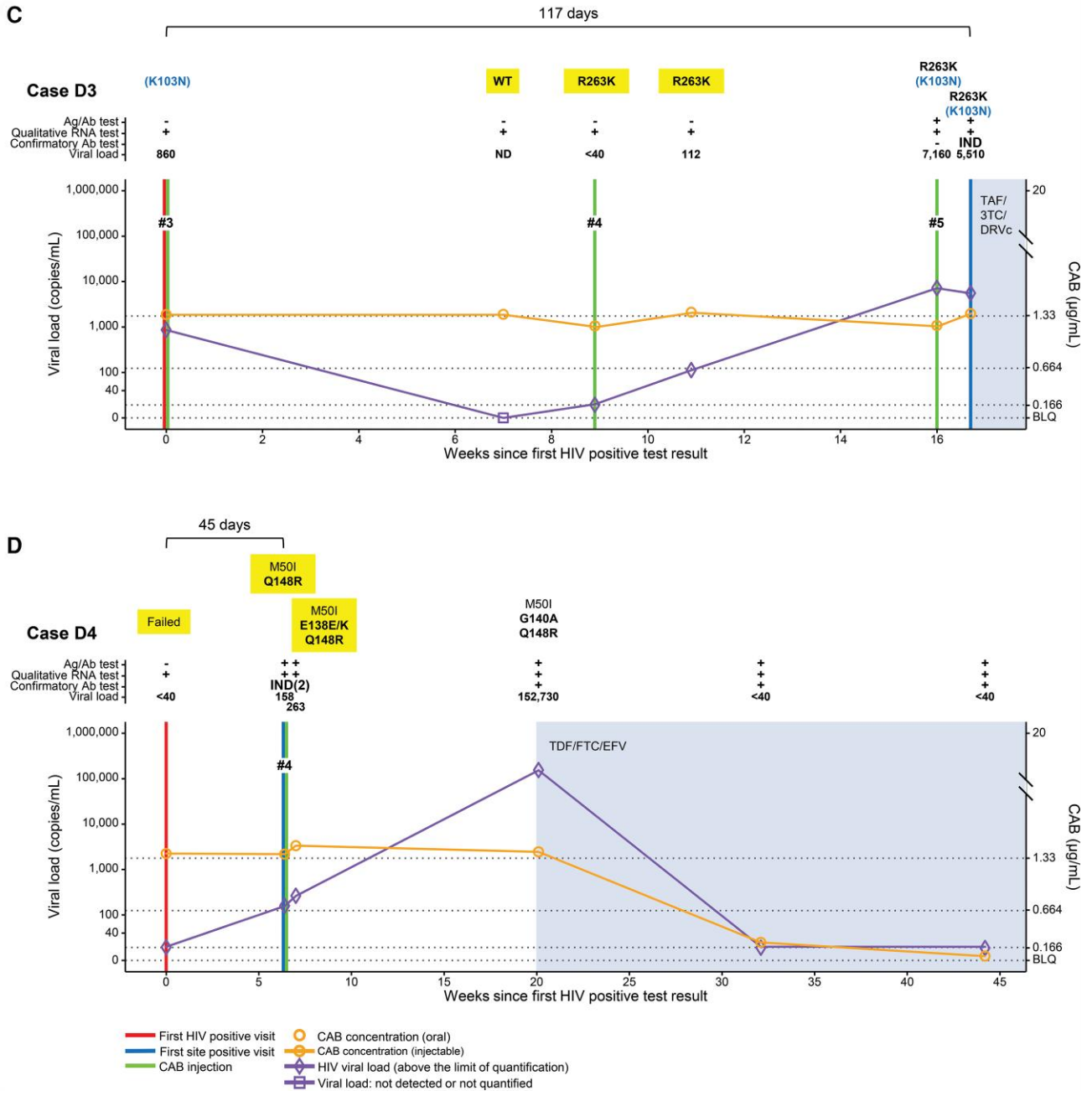

Figure 4. Continued

detect both RNA and DNA may not offer benefit over RNA assays in this setting, because HIV DNA was often undetectable or present at low levels in this setting [3, 4]. Studies are planned to evaluate performance of additional assays for detection of HIV infection in challenge samples from HPTN 083.

Only 2 participants in the HPTN 083 CAB arm who acquired HIV infection in the blinded phase of the trial received initial ART with an INSTI-based regimen (cases B4 and B5); neither of those participants received CAB in the 6 months before the first HIV positive visit and neither had INSTI resistance. More data are needed to assess the performance of dolutegravir- or bictegravir-based ART in infections that occur in the setting of CAB PrEP. The infections that presented in this report occurred before or during CAB-LA dosing. Studies are underway to assess INSTI resistance in infections that occur in the CAB-LA tail phase (after the last injection); INSTI RAMs were not detected in preliminary analysis of 3 tail-phase infections [17].

Table 1. Summary of cases with INSTI RAMs detected.

Clinical presentation	Case	HIV ST	Number of injections		Detection delay (days)	Viral load (copies/mL)		INSTI RAMs		
			Total	After infection		1st HIV pos visit	1st visit with major INSTI RAMs	1st HIV pos visit	1st site pos visit	Subsequent visits[a]
Infected before CAB	A2	C	1	1	60	50,080	204	None	M50I, **E138K, Q148K**	M50I, **E138K, Q148K**
Infected while receiving oral CAB	C1	B	2	2	47	120	137	L74I, Q146Q/R, E157Q	L74I, **E138E/K, G140G/S, Q148R,** E157Q	L74I, **E138E/K, G140G/S, Q148R,** E157Q
	C3	B	1	1	35	15.3[b]	102,329	None	**E138A, Q148R**	Not tested/ on ART
Infected while receiving CAB injections	D1	B[c]	10	3	112	130	130	**N155H**	**N155H, R263K**[d]	Not tested/ on ART
	D2	B[c]	6	2	98	6.1[b]	<40	N/A	N/A	**N155H,** S230R
	D3	B/F	5	3	117	860	<40	None[e]	**R263K**	Not tested/ on ART
	D4	C	4	1	45	<40	158	N/A	M50I, **Q148R**	M50I, **E138E/K, G140A, Q148R**

[a]This column shows additional INSTI RAMs that were detected at visits after the first site positive visit.

[b]These viral load results were obtained using a single copy HIV RNA assay.

[c]In these two cases, the most likely HIV subtype was determined based on the location of the study sites.

[d]In this case, an accessory mutation (Q146L) and a major INSTI RAM (Q148R) were also detected between the first HIV positive visit and the first site positive visit.

[e]In this case, the non-nucleoside reverse transcriptase (NNRTI) mutation, K103N, was detected at the first HIV positive visit.

Abbreviations: ART, antiretroviral treatment; CAB, cabotegravir; HIV, human immunodeficiency virus; INSTI, integrase strand transfer inhibitor; NA, data not available (no genotyping results obtained); RAM, resistance-associated mutation; ST, subtype.

Characteristics of cases with INSTI RAMs detected. The first HIV positive visit was identified based on results of retrospective HIV RNA testing performed at the HPTN Laboratory Center. The first site positive visit is the first visit where the participant had a reactive rapid test or a reactive antigen/antibody test at the study site. Major INSTI RAMs are shown in bold text.

This study has some limitations. First, because of the small number of HIV infections in HPTN 083, there were few cases available for analysis; these findings may not be generalizable to all cases of CAB PrEP-associated infections. Second, a limited volume of plasma was available for testing with the low VL SGS-IN assay, results were not obtained for 3 samples with very low viral loads, and the number of sequence reads obtained for some samples was low. These factors may have impacted detection of INSTI RAMs using this assay. Third, this study was limited to analysis of INSTI resistance in cisgender men and

Table 2. Characteristics of the first visit with a major INSTI RAM detected.

Timing of infection	Case	Viral load (copies/mL)	Major INSTI RAMs detected	Timing			CAB concentration (μg/mL)[a]
				Relative to injections	Relative to the 1st site pos visit	Days since the 1st HIV pos visit	
Enrollment	A2	204	**E138E/K, Q148K/R**	1st injection visit	Before	40	3.318
While receiving oral CAB	C1	137	**Q148Q/R**	8 days after the 1st injection	Before	17	1.219
	C3	102,329	**E138A, Q148R**	20 days after the 1st injection	Same visit	35	1.108
While receiving CAB injections	D1	130	**N155H**	8th injection visit	Before	0	1.613
	D2	<40	**N155H**	182 days after the 6th injection	After[b]	280[b]	0.703
	D3	<40	**R263K**	4th injection visit	Before	62	1.213
	D4	158	**Q148R**	4th injection visit	Same visit[c]	45	1.930

[a]The concentration of cabotegravir associated with protection against HIV infection (PAIC90) is 1.33 μg/mL.

[b]In these cases, HIV genotyping results were not obtained prior to the first site positive visit.

[c]Only three of 11 visits during this interval had a positive result with the qualitative RNA assay; two samples from this case failed genotyping.

Abbreviations: CAB, cabotegravir; HIV, human immunodeficiency virus; INSTI, integrase strand transfer inhibitor; RAM, resistance associated mutation.

Characteristics of the first visit with major INSTI RAMs detected. The first HIV positive visit was identified based on results of retrospective HIV RNA testing performed at the HPTN Laboratory Center. The first site positive visit is the first visit where the participant had a reactive rapid test or a reactive antigen/antibody test at the study site.

transgender women who have sex with men. CAB pharmacokinetics are different in individuals assigned male at birth compared to individuals assigned female at birth [18], which could impact emergence of INSTI resistance in the setting of CAB-LA PrEP. Fourth, most of the participants in HPTN 083 were enrolled in the United States and Latin America, where most infections are likely to be caused by HIV subtype B. The 7 cases reported here included 4 with subtype B infection, 1 with B/F recombinant HIV, and 2 with subtype C infection. HIV subtype can impact the emergence and fading of drug-resistant HIV variants [19], and may impact emergence of INSTI resistance in persons receiving CAB-LA PrEP.

While this report supports use of HIV RNA screening in the setting of CAB-LA PrEP, it is important to put this information in context. In primary analysis of HPTN 083, only 16 HIV infections were identified among 2282 participants in the CAB arm with >3000 person-years of follow-up. Only 7 of the 16 cases identified during the blinded phase of the trial had INSTI RAMs detected; 1 of those participants would not have received CAB if the infection had been detected prior to study enrollment and 2 others were infected while receiving oral CAB, which is considered optional by the US FDA prior to starting CAB-LA PrEP. There is also an urgent need for effective methods for HIV prevention that do not rely on daily pill taking. For these reasons, we believe that CAB-LA PrEP should be implemented regardless of the availability or feasibility of HIV RNA screening. Broad implementation of prevention services that include CAB-LA PrEP is critical for protecting the high-risk populations for which CAB-LA PrEP was developed. In settings where routine RNA screening is not feasible, evaluation of the impact of CAB-LA implementation on INSTI resistance will help inform health policy. Further studies are also needed to identify optimal ART regimens for treatment of CAB-LA PrEP breakthrough infections.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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Author contributions. All authors participated in the study, contributed to manuscript preparation, and reviewed the manuscript. S. E. (HPTN 083 protocol virologist) designed the study, analyzed data, and drafted the manuscript. J. M. F. (HPTN laboratory center virologist) analyzed data and drafted the manuscript. E. H. tested and interpreted results from low viral load INSTI genotyping. E. P.-M. (HPTN laboratory center deputy director) coordinated laboratory testing. M. A. M. (lead laboratory pharmacologist) designed the study, analyzed data, and drafted the manuscript. R. K. provided graphic arts support. Z. W. (HPTN SDMC data analysis) provided data management and analysis. J. M. provided input on HIV virology. M. M. HPTN 083 study coordinator. A. R. R. and M. S.-C. provided pharmaceutical support. A. A. HPTN 083 NIH medical officer. J. C. H., R. C., and K. M. HPTN 083 site PI. B. H. HPTN 083 statistician. M. C. HPTN leadership and operations center PI. B. G. HPTN 083 protocol cochair. R. L. (HPTN 083 protocol chair) designed the study, analyzed data, and drafted the manuscript.

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Potential conflicts of interest. R. J. L. has served on scientific advisory boards for Merck and has received honoraria from Roche and Janssen. A. R. and M. S. C. are employees of ViiV Healthcare. J. W. M. is a consultant to Gilead Sciences, owns shares of Abound Bio, Inc, and share options in Infectious Disease Connect. All other authors report no potential conflicts.

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References

1. Landovitz RJ, Donnell D, Clement ME, et al. Cabotegravir for HIV prevention in cisgender men and transgender women. N Engl J Med **2021**; 385:595–608.
2. ViiV Healthcare Limited. Apretude (package insert). https://www.accessdata.fda.gov/drugsatfda_docs/label/2021/215499s000lbl.pdf. Accessed 18 February 2022.

3. Marzinke MA, Grinsztejn B, Fogel JM, et al. Characterization of human immunodeficiency virus (HIV) infection in cisgender men and transgender women who have sex with men receiving injectable cabotegravir for HIV prevention: HPTN 083. J Infect Dis **2021**; 224: 1581–92.
4. Eshleman SH, Fogel JM, Piwowar-Manning E, et al. Characterization of human immunodeficiency virus (HIV) infections in women who received injectable cabotegravir or tenofovir disoproxil fumarate/emtricitabine for HIV prevention: HPTN 084. J Infect Dis **2022**; 225:1741–9.
5. World Health Organization. Consolidated guidelines on HIV prevention, testing, treatment, service delivery and monitoring: recommendations for a public health approach, **2021**. https://www.who.int/publications/i/item/9789240031593. Accessed 18 February 2022.
6. US Department of Health and Human Services. Guidelines for the use of antiretroviral agents in adults and adolescents living with HIV, **2022**. https://clinicalinfo.hiv.gov/en/guidelines/adult-and-adolescent-arv/whats-new-guidelines. Accessed 18 February 2022.
7. Tosiano MA, Jacobs JL, Shutt KA, Cyktor JC, Mellors JW. A simpler and more sensitive single-copy HIV-1 RNA assay for quantification of persistent HIV-1 viremia in individuals on suppressive antiretroviral therapy. J Clin Microbiol **2019**; 57:e01714-18.
8. Stanford University. HIV drug resistance database. https://hivdb.stanford.edu. Accessed 18 February 2022.
9. Palmer S, Kearney M, Maldarelli F, et al. Multiple, linked human immunodeficiency virus type 1 drug resistance mutations in treatment-experienced patients are missed by standard genotype analysis. J Clin Microbiol **2005**; 43: 406–13.
10. Halvas EK, Joseph KW, Brandt LD, et al. HIV-1 viremia not suppressible by antiretroviral therapy can originate from large T cell clones producing infectious virus. J Clin Invest **2020**; 130:5847–57.
11. Margolis DA, Gonzalez-Garcia J, Stellbrink HJ, et al. Long-acting intramuscular cabotegravir and rilpivirine in adults with HIV-1 infection (LATTE-2): 96-week results of a randomised, open-label, phase 2b, non-inferiority trial. Lancet **2017**; 390:1499–510.
12. Andrews CD, Spreen WR, Mohri H, et al. Long-acting integrase inhibitor protects macaques from intrarectal simian/human immunodeficiency virus. Science **2014**; 343: 1151–4.
13. US Public Health Service. Preexposure prophylaxis for the prevention of HIV infection in the United States—2021 update. https://www.cdc.gov/hiv/pdf/risk/prep/cdc-hiv-prep-guidelines-2021.pdf. Accessed 18 February 2022.
14. European AIDS Clinical Society. EACS guidelines 2022. https://eacs.sanfordguide.com/. Accessed 28 October 2022.
15. Stekler JD, McKernan J, Milne R, et al. Lack of resistance to integrase inhibitors among antiretroviral-naive subjects with primary HIV-1 infection, 2007–2013. Antivir Ther **2015**; 20:77–80.
16. Mbisa JL, Ledesma J, Kirwan P, et al. Surveillance of HIV-1 transmitted integrase strand transfer inhibitor resistance in the UK. J Antimicrob Chemother **2020**; 75:3311–8.
17. Landovitz RJ, Donnell D, Tran H, et al. Updated efficacy, safety and case studies in HPTN 083: CAB-LA vs TDF/FTC for PrEP. Conference on Retroviruses and Opportunistic Infections, 12–16 February **2022**. Abstract LB96. https://www.croiconference.org/abstract/updated-efficacy-safety-and-case-studies-in-hptn-083-cab-la-vs-tdf-ftc-for-prep/. Accessed 28 October 2022.
18. Landovitz RJ, Li S, Grinsztejn B, et al. Safety, tolerability, and pharmacokinetics of long-acting injectable cabotegravir in low-risk HIV-uninfected individuals: hPTN 077, a phase 2a randomized controlled trial. PLoS Med **2018**; 15:e1002690.
19. Eshleman SH, Guay LA, Wang J, et al. Distinct patterns of emergence and fading of K103N and Y181C in women with subtype A vs. D after single-dose nevirapine: HIVNET 012. J Acquir Immune Defic Syndr **2005**; 40: 24–9.